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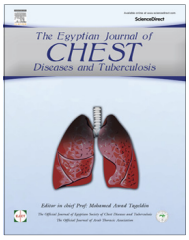

ORIGINAL ARTICLE

Tumor necrosis factor-alpha and CD4/CD8 ratio in patients with hypersensitivity pneumonitis

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Abstract Hypersensitivity pneumonitis (HP) is an immunologically induced lung disease caused by inhalation of a variety of environmental agents. HP is not a uniform disease but rather a complex syndrome characterized by varying intensities of responsiveness to different antigens leading to an immunopathology with variable clinical presentation and natural history. We studied the association between serum TNF-alpha and CD4/CD8 ratio with chest computed tomography findings and steroid responsiveness.

Methods: The study included 46 patients with chronic hypersensitivity pneumonitis, 22 (47.8%) male and 24 (52.8%) female. mean age 49 ± 8 years. All patients underwent high resolution chest computed tomography, TNF-alpha ELIZA assay, and cd3, cd4, cd8, CD4/CD8 by flow cytometry.

Results: The TNF-alpha level was (mean ± SD) 299 ± 427 pg/ml, CD4/CD8 ratio (84 ± 36). With regard to steroid responsiveness (26) 57% patients were steroid responders while 20 (43%) were non-responders.

TNF-alpha level was significantly lower in patients with predominant ground glass in their chest computed tomography ($p = 0.014$), however CD3, CD4, CD8, CD4/CD8 levels showed insignificant differences between patients with ground glass and those with fibrosis in their chest computed tomography.

Finally, TNF-alpha level was significantly lower in patients with good steroid response ($p = 0.014$), on the other hand CD4/CD8 ratio was significantly higher in those with good steroid response ($P = 0.011$).

Conclusion: Low TNF-alpha and high CD4/CD8 ratio could be used as a predictor of steroid responsiveness in CHP patients.

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Introduction

Extrinsic allergic alveolitis (EAA) belongs to the family of interstitial lung diseases. It results from repeated inhalation of causative antigens in susceptible people. The prevalence of the disease is unknown. It is probable that both humoral and cellular mechanisms participate in the development of the lung lesion after the repeated inhalation of offending antigens. For the acute form of the disease, which occurs several hours after the antigen challenge, an immune complex mediated tissue injury is typical [1,2].

On the other hand, T cell mediated immune inflammatory response prevails in advanced stages of the disease [3].

The clinical presentation of the disease has been defined as acute, subacute, and chronic. According to recent knowledge, the disease could be retrospectively classified as acute intermittent, acute progressive, chronic progressive, and chronic nonprogressive [4].

Exposure to known sensitizing antigens is the most important diagnostic sign [4]. The typical high resolution computed tomography (HRCT) findings, bronchoalveolar lavage (BAL), and in some cases the surgical lung biopsy are also important for diagnosis of EAA [5].

Hypersensitivity pneumonitis (HP) is an immunologically induced lung disease caused by inhalation of a variety of environmental agents [6]. HP is not a uniform disease but rather a complex syndrome characterized by varying intensities of responsiveness to different antigens leading to an immunopathology with variable clinical presentation and natural history [7]. The long-term outcome of bird fancier's lung and farmer's lung appears to be variable, including persistent obstructive airways disease [8–10]. Continued exposure to antigen does not consistently lead to clinical deterioration, except in some patients such as those with pigeon breeder's disease. Patients with pigeon breeder's disease and farmer's lung frequently progress to pulmonary fibrosis despite avoidance of exposure to pigeon antigens [8,9,11]. Development of pulmonary fibrosis appears to depend on the immune responses of individual patients and/or the biologic characteristics of the causative antigen. Bronchoalveolar lavage (BAL) has revealed many characteristic aspects of cellular and humeral components of HP. It is widely accepted that predominant CD8 + T lymphocyte alveolitis is a feature of HP at an acute phase. However, in several cases, especially at a chronic phase, pulmonary fibrosis may be another feature of radiologic findings of HP, and CD8 + T-cell dominance in BAL fluids may disappear [12,13].

Tumor necrosis factor (TNF)-α is produced primarily by activated macrophages [14,15]. It is viewed as a pleiotropic cytokine with broad immune regulation functions and is closely related to the development of diseases [16,17] TNF-α exerts its diverse biological effects by binding to two specific cell surface receptors (membrane TNF receptor [mTNFR]-1 and mTNFR-2) [18,19], which are expressed on a variety of cells [19–21]. Both receptors can be shed from the cell surface to form soluble TNF receptor (sTNFR)-1 and sTNFR-2 [20–25].

Patients

A single center observational study was carried out in the chest department, Assuit University hospital, Assuit Egypt (Tertiary hospital for all upper Egypt Governorates) between January 2013 and December 2014. The study included 46 patients, 22 (47.8%) male and 24 (52.8%) female.

All patients with chronic hypersensitivity pneumonitis (CHP), admitted during the study period to Chest department at Assuit University, who agreed to participate in the research, were included in this study. The diagnosis of CHP was made based on history of exposure, clinical examination, high-resolution computerized tomography (HRCT) of the chest and pulmonary function testing (PFT). None of the cases accepted to confirm the diagnosis by either thoracoscopic lung biopsy or transbronchial lung biopsy. The presence of typical clinical and HRCT features of CHP, when identified by expert clinicians and radiologists, is sufficiently characteristic to allow a confident diagnosis and eliminate the need for surgical lung biopsy. All cases had also abnormal pulmonary function studies including evidence of restriction—reduced vital capacity with increased FEV1/FVC ratio. There was no evidence of either coexisting collagen-vascular disease.

Steroid responsiveness was assessed according to clinical, radiographic, and physiologic scoring system for the longitudinal assessment of patients with idiopathic pulmonary fibrosis [26,27].

The study was approved by the Re-search Ethics Committee Assuit Faculty of Medicine, Assuit university.

Methodology

Sample collection and laboratory investigations

A 5-ml sample of venous blood was collected from both patient and control groups by venipuncture under completely aseptic conditions. The samples were collected in two types of tubes:

1. 3 ml of venous blood in plain tubes and were allowed to clot at room temperature for at least 30 min, then centrifuged at 1500 rpm for 15 min at room temperature to get serum samples. Serum samples were divided and stored in aliquots at −20 °C until analyzed.
2. 2 ml of venous blood in K3EDTA anticoagulant tubes for complete blood count (CBC) and Flowcytometic analysis.

Assay of serum levels of TNF-α was performed using AviBion Human TNF-α ELISA Kit (Orgenium, Finland, Rev 02.10) according to the manufacturer.

CBC for patients was done by Cell-Dyne 3700 Appott *USA* according to the manufacturer and absolute lymphocytic count was determined for patients.

Flowcytometry analysis

T- Lymphocyte subsets in whole blood samples were enumerated using fluoroisothiocyanate (FITC)-conjugated CD 4 (Becto + Dickinson, Bioscience, USA), phycoerythrin (PE) conjugated CD8 (Becton Dickinson, Bioscience, USA) and peridinium-chlorophyll-protein (Per-CP)-conjugated CD3 (Becton Dickinson, Bioscience, USA). 100 µl of blood sample was incubated with 20 µl of CD4, CD8, CD 3 triple color for 15 min at room temperature in the dark followed by incubation, RBC lysis, and washing with phosphate buffered saline (PBS). After one wash, the cells were resuspended in PBS.

Flow cytometric analysis was done by FACS Calibur flow cytometry with CellQuest software (Becton Dickinson Biosciences, USA). An isotype-matched negative control was used with each sample. Forward and side scatter histogram was used to define the lymphocyte population (R1). % of CD 3 positive cells refers to the total T Lymphocytes; CD 4 positive cells are the helper cells and CD8 positive are the cytotoxic cells.

Radiologic assessment

HRCT images were obtained on a variety of scanners using 1- to 2-mm collimation with 10- to 20-mm spacing and a high-spatial frequency reconstruction algorithm. An experienced thoracic radiologist who was blinded to all clinical data reviewed the CT scans. The radiologist assessed the scans for the presence or absence of parenchymal fibrosis. Parenchymal fibrosis was defined as the presence of irregular linear opacities, traction bronchiectasis, or honeycombing [27].

Results

The current study included 46 patients with CHP, 22 male and 24 female, mean age 49 ± 8 years. Two patients were current smokers, 8 ex-smokers and 36 patients were non-smokers (Table 1).

Ten (22%) patients were bird breeders, 8 patients (17%) were employers, 12 (26%) farmers and 16 (35%) housewives (Fig. 1).

Chest computed tomography evaluation of 46 patients with CHP showed that 26 (56%) patients have more than 30% areas of ground glass, as evaluated by two independent radiologist, while 20 (44%) of patients have less than 30% ground glass, fibrosis or reticulonodular pattern was the predominant feature in their chest computed tomography (Fig. 2).

The TNF-alpha level was (mean ± SD) 299 ± 427 pg/ml, CD4/CD8 ratio (84 ± 36). With regard to steroid responsiveness 26 (57%) patients were steroid responders while 20 (43%) were non-responders (Fig. 3).

TNF-alpha level was significantly lower in patients with predominant ground glass in their chest computed tomography ($p = 0.014$), however CD3, CD4, CD8, CD4/CD8 levels showed insignificant differences between patients with ground glass and those with fibrosis in their chest computed tomography (Tables 2–4).

Finally, TNF-alpha level was significantly lower in patients with good steroid response ($p = 0.014$), on the other hand CD4/CD8 ratio was significantly higher in those with good steroid response ($P = 0.011$).

Table 1 Demographic data of study population.

Age (mean ± SD)	49 ± 8.7
Sex	
Male (%)	22 (48)
Female (%)	24 (52)
Smoking status	
Current smoker (%)	2 (4)
Ex-smoker (%)	8 (17)
Non-smoker (%)	36 (79)

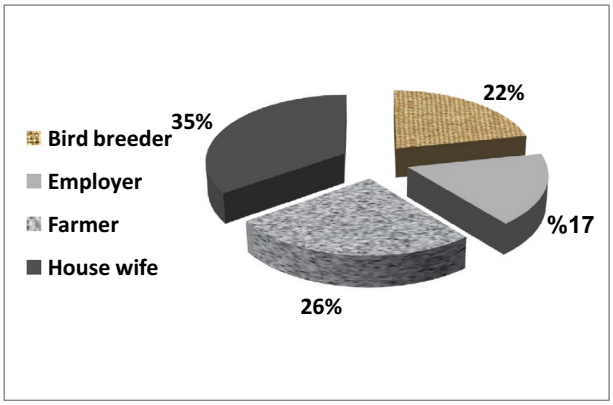

Figure 1 Occupation of study population.

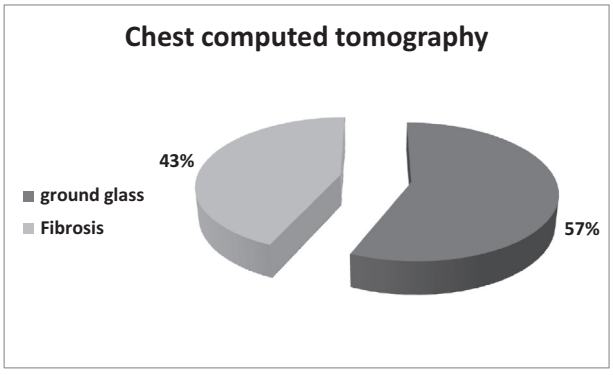

Figure 2 Chest computed tomography pattern.

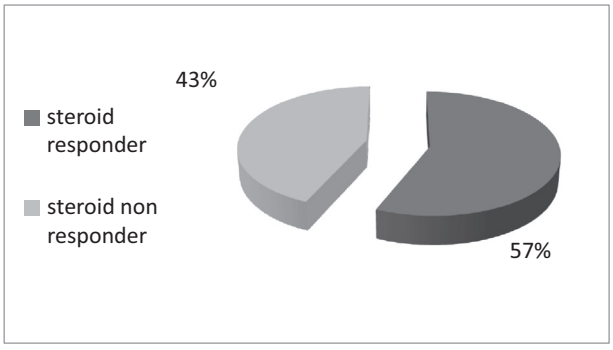

Figure 3 Response to corticosteroid.

Discussion

In the current study we found that, TNF-alpha is negatively associated with response to corticosteroid treatment in patients with CHP, and it was associated with more fibrosis in chest computed tomography of these patients. On the other hand the CD4/CD8 ratio was more positively correlated with steroid response in patients with CHP, also, ground glass pattern on chest computed tomography was significantly correlated with steroid response.

EAA is a disease characterized by pulmonary inflammation and granuloma formation. Alveolar macrophage (AM) and AM-derived cytokines, in particular TNF-alpha, play a crucial

Table 2 CD3, CD4, CD8, CD4/CD8 and TNF-alpha according to chest computed tomography.

	Fibrosis	Ground glass	*P* value
CD3 (Mean ± SD)	4 ± 4	5 ± 6	0.695
CD4 (Mean ± SD)	2 ± 2	2 ± 3	0.825
CD8 (Mean ± SD)	2 ± 2	3 ± 4	0.488
CD4/CD8 (Mean ± SD)	84 ± 42	84 ± 32	0.998
TNF-α (Mean ± SD)	540 ± 486	114 ± 267	0.014

Table 3 CD3, CD4, CD8, CD4/CD8 and TNF-alpha according response to corticosteroid.

	Steroid non responder	Stroid responder	*P* value
CD3 (Mean ± SD)	2.4 ± 1.5	5.5 ± 6.7	0.223
CD4 (Mean ± SD)	0.9 ± 0.7	2.5 ± 3	0.17
CD8 (Mean ± SD)	1.5 ± 1	3 ± 3.7	0.313
CD4/CD8 (Mean ± SD)	59 ± 15	98 ± 37	0.011
TNF-α (Mean ± SD)	540 ± 486	114 ± 267	0.014

Table 4 Steroid response according to chest computed tomography.

	CT-Chest		
Steroid response	Ground glass	Fibrosis	*P*-value
Responder (26)	20	6	0.04
Non-responder (20)	6	14	

role in these processes. TNF-alpha, spontaneously released by activated AMs in EAA, can enhance the production of interleukin-1, interleukin-6, monocyte-chemoattractant protein-1, and macrophage inflammatory protein-1alpha [16,17], all mediators that have been shown to be involved in the development of EAA [28–31]. TNF-alpha can promote cellular recruitment and activation in the lungs and thus contribute to the alveolitis characterized by infiltration of mononuclear cells and the formation of granulomas [28–30].

Previous studies have shown that the CD4/CD8 ratio in BAL fluid may vary significantly according to the clinical presentation of the disease, the biological characteristics of the inhalation antigen, the exposure to the causative antigen at the time of the diagnosis, the immune susceptibility of particular patients, and to the history of smoking [32]. Our data support the hypothesis of the correlation of the continuous exposure to the inhalation antigen and the low CD4/CD8 ratio in BAL fluid. Murayama raises the possibility that increased CD8 + T cells might have a protective effect against pulmonary fibrosis while comparatively increased CD4 + T cells might play an important part in the pathogenesis of pulmonary fibrosis in EAA [33]. We proved that the persons with the normal CD4/CD 8 ratio had higher interstitial scores.

Mornex et al." reported that CD4 + cell-dominant T lymphocytosis was observed in BAL fluids of HP when the exposure to antigens had been avoided for more than five days. Although CD4 + cell-dominant T lymphocytosis of the fibrosis group is similar to those patients after the avoidance of exposure, our observation regarding T cells in BAL fluids was obtained during continuous exposure to the antigen.

The chronic stage of HP has been extensively characterized in the farmer's lung, [34] in which the principal findings were interstitial pulmonary fibrosis and CD4 + T-cell dominance in BAL fluids in several cases. In addition, the chronic stage of bird fancier's lung has been reported to develop pulmonary fibrosis and have CD4 + T-cell-dominant BAL findings [35].

Conflict of interest

The authors declare that they have no conflict of interest.

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